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^{18}Fluorodeoxyglucose Positron Emission Tomography Imaging of Atherosclerotic Plaque Inflammation Is Highly Reproducible

Implications for Atherosclerosis Therapy Trials

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Objectives This study tested the near-term reproducibility of ^{18}fluorodeoxyglucose positron emission tomography (FDG-PET) imaging of atherosclerosis.

Background It is known that FDG-PET can measure inflammation within the aorta, carotid, and vertebral arteries with histologic validation in humans and animal models of disease. By tracking changes in inflammation over time, PET could be used as a surrogate marker of antiatheroma drug efficacy. However, the short-term variability and reproducibility of the technique are unknown.

Methods We imaged the carotid arteries and aorta in 11 subjects with FDG-PET/computed tomography twice, 14 days apart. We assessed interobserver and intraobserver agreement and interscan variability.

Results Interscan plaque FDG variability over 2 weeks was very low; intraclass correlation coefficients (ICC) ranged between 0.79 and 0.92. Interobserver agreement was high across all territories imaged except aortic arch (ICC values from 0.90 to 0.97, arch 0.71). Intraobserver agreement was high, with ICC values between 0.93 and 0.98.

Conclusions Spontaneous change in plaque FDG uptake is low over 2 weeks, with favorable inter- and intraobserver agreement. Power calculations suggest that drug studies using FDG-PET imaging would require few subjects compared with other imaging modalities. This study strengthens the case for FDG-PET as a noninvasive plaque imaging technique. (J Am Coll Cardiol 2007;50:892–6) © 2007 by the American College of Cardiology Foundation

Atherosclerosis is a global epidemic and likely to become the leading cause of death worldwide by 2010. Clinical events attributable to plaque rupture are related to the level of plaque inflammation.

Nevertheless, several drugs are expected to improve the outlook over the next decade. Unfortunately, testing new treatments requires large trials with clinical end points. Therefore, drug developers need early markers of drug efficacy before beginning costly trials. The use of imaging for this role has been suggested (1).

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^{18}Fluorodeoxyglucose (FDG) positron emission tomography (PET) is firmly established in oncology for monitoring the response of tumors to treatment (2), and it was shown in 2002 (3) that FDG-PET imaging could image metabolic activity within carotid atherosclerosis as a marker of plaque inflammation. Others have expanded on this work by imaging the vertebral arteries (4) and aorta (5). Tawakol (6) showed a positive correlation between carotid plaque FDG uptake and macrophage content. Animal work (7) demonstrated that plaque inflammation can be reduced by probucol and quantified using FDG-PET. In the first human study of its kind (8), FDG-PET was used to monitor reduction in carotid plaque inflammation during statin therapy.

However, more information is still required. First, the near-term reproducibility of plaque PET/computed tomography (CT) imaging is unknown. Second, both interobserver and intraobserver agreements have not been tested. Finally, we need to define the mean and standard deviation

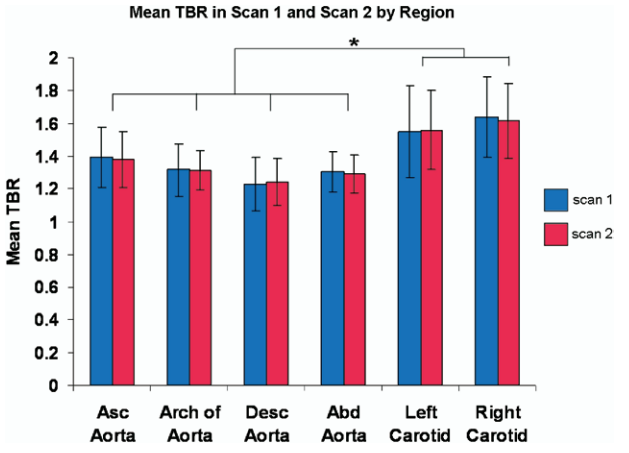

Figure 1 Mean TBR Values at First and Second Scan

Error bars indicate standard deviation. Little spontaneous change in tissue-to-background ratio (TBR) values between the two scans is noted. *$p < 0.05$ for the TBR of both left and right carotid artery compared with the TBR of all aortic regions. Abd = abdominal; Asc = ascending; Desc = descending.

of plaque FDG measurements in different vascular beds. This knowledge will allow the calculation of sample sizes for adequately powered drug studies.

We tested the interscan variability of FDG-PET imaging by scanning 11 subjects twice within 2 weeks and measuring the interobserver and intraobserver agreement of the technique.

Methods

Study population. We recruited 11 subjects with established vascular disease or elevated Framingham risk scores from Mount Sinai Medical Center. Subjects gave written consent, and the study was approved by the local Institutional Review Board.

PET/CT imaging. Subjects underwent 2 PET/CT scans 2 weeks apart (scan 1 and scan 2) on a GE (Milwaukee, Wisconsin) Lightspeed PET/CT scanner. Subjects with a prescan glucose level of ≥200 mg/dl were excluded. Fluorodeoxyglucose was injected, and patients rested for 90 min. Aortic imaging was performed first, with a CT scan for localization and attenuation correction. The aortic arch was the upper limit of the scan, which covered 3 bed positions in 2-dimensional (2D) mode for 10 min each. Next, subjects were placed into a head holder, and a single-bed position carotid PET scan was performed in 3-dimensional (3D) mode for 15 min. For carotid imaging, the scanner was found to perform best in 3D mode compared to 2D mode, giving images of greater uniformity, resolution, and sensitivity. However, when 3D-mode imaging of the aorta was attempted, significant signal dropout artifacts were sometimes noted, probably because of high FDG uptake in adjacent structures causing dead-time issues. Therefore, we reverted to 2D-mode aortic imaging.

Abbreviations and Acronyms

CT = computed tomography
FDG = ^{18}fluorodeoxyglucose
ICC = intraclass correlation coefficient
MRI = magnetic resonance imaging
PET = positron emission tomography
ROI = region of interest
SUV = standardized uptake value
TBR = tissue-to-background ratio
2D = 2-dimensional
3D = 3-dimensional

Image analysis. Image analysis was performed on a Xeleris workstation. The aorta was divided into ascending, arch, and descending segments using CT images. Arterial FDG uptake was quantified by drawing a region of interest (ROI) around each artery on every slice of the coregistered transaxial PET/CT images. Next, the arterial standardized uptake value (SUV) was calculated as the mean pixel activity within the ROI.

By averaging the SUV values for each artery slice, we derived a mean SUV value for the entire artery (arterial SUV). This was corrected for blood activity by division by the average blood SUV estimated from either the inferior vena cava or jugular vein to produce a blood-corrected artery SUV, known as the arterial tissue-to-background ratio (TBR) (6).

Testing intraobserver and interobserver agreement. An experienced reader (J.R.) analyzed all studies (scan 1 and scan 2) in every subject. Additionally, intraobserver agreement was assessed. Scan 1 studies of all 11 subjects were read a second time by the same reader. To reduce recall bias, the second reading took place at least 1 month after the first reading. After a period of joint working on pilot images (not included in this study) to establish methods of analysis and recording, interobserver agreement was tested. All 11 patients' first PET scans (scan 1) were independently read by another reader (K.M.). The studies were presented in a random order.

Statistical methods. Continuous variables are expressed as mean ± SD. Paired 2-sided *t* tests were used to check for

Table 1 TBR Variability Over 2 Weeks

	Ascending Aorta	Arch of Aorta	Descending Aorta	Abdominal Aorta	Left Carotid	Right Carotid
Scan 1 TBR	1.39 (0.18)	1.31 (0.16)	1.23 (0.16)	1.30 (0.12)	1.55 (0.28)	1.64 (0.25)
Scan 2 TBR	1.38 (0.17)	1.31 (0.12)	1.24 (0.14)	1.29 (0.12)	1.56 (0.24)	1.62 (0.23)
TBR difference	0.01 (0.07)	0.0005 (0.06)	−0.084 (0.10)	0.01 (0.08)	0.0097 (0.12)	0.0201 (0.11)
95% normal range for change	0.14	0.12	0.20	0.16	0.24	0.22

Mean (SD). Tissue-to-background ratio (TBR) values for scan 1 and 2 and the differences and 95% normal ranges for 'allowable' change in TBR between scans over a 2-week period.

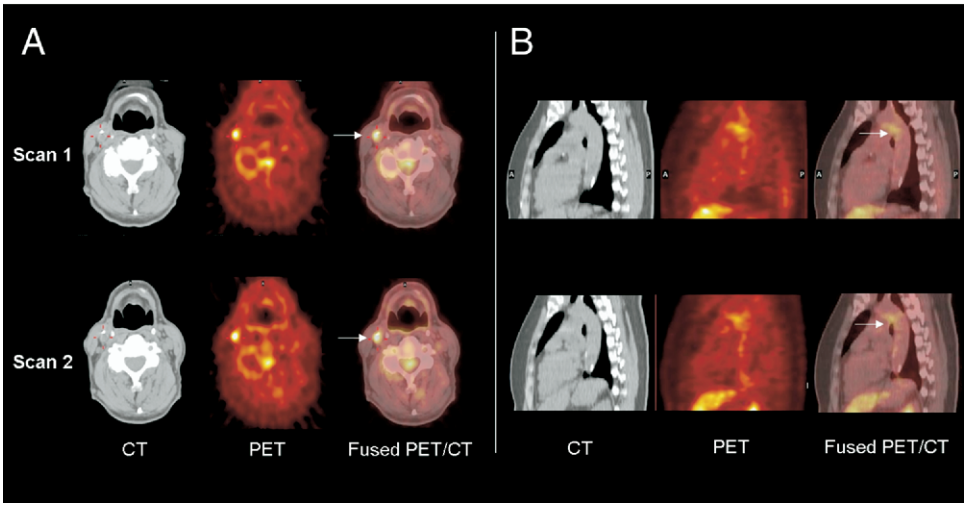

Figure 2 Carotid and Aortic PET/CT Images Over 2 Weeks

(A) Axial images from scan 1 and 2 of one subject's carotid artery. Computed tomography (CT) **(left)**, positron emission tomography (PET) **(middle)**, and fused PET/CT **(right)** with **arrows** indicating similar ^{18}fluorodeoxyglucose (FDG) uptake in the right carotid artery at both time points. **(B)** Sagittal images from scan 1 and 2 of another subject's aorta. CT **(left)**, PET **(middle)**, and fused PET/CT **(right)** with arrows indicating similar FDG uptake in the aortic arch and descending aorta at both time points.

differences between mean values of continuous variables. In this study, corrections for multiple comparisons were not applied. The 95% normal ranges for TBR differences between scans 1 and 2 were estimated by doubling the standard deviation of the mean difference between the 2 scans (9). A p value of <5% was considered statistically significant.

Power analyses were based on a 2-sample unpaired *t* test (2-sided) and performed with 80% power and an alpha of 5%.

Intraclass correlation coefficients (ICC) (10) with 95% confidence intervals were calculated to test the interscan variability and also to assess interobserver and intraobserver agreement. An ICC value of 1 indicates perfect agreement, with random or systematic differences between the 2 measurements decreasing the value.

Bland-Altman plots were also used to assess interobserver, intraobserver, and interscan variability. Statistical analysis was performed using SPSS version 14 (SPSS Inc., Chicago, Illinois).

Results

Patient characteristics. Eleven asymptomatic patients were imaged twice, 14 days apart. Mean age was 64.6 ± 7.3 years, and there were 4 women. All patients had atherosclerosis or elevated risk scores (2 previous transient ischemic attack, 8 coronary artery disease with prior revascularization, and 1 subject with prior myocardial infarction). Five subjects had type 2 diabetes.

Imaging parameters. The FDG doses were similar across both scans (mean scan 1: 572 ± 54 MBq, mean scan 2: 592 ± 46 MBq, p = 0.27). Aortic imaging commenced 96.6 ± 12.3 min after injection and was not different between scans (mean scan 1: 95.7 ± 10.6 min, mean scan 2: 97.5 ± 14.3 min, p = 0.37). Similarly, mean start time for carotid imaging was 145.7 ± 16.1 min, with no significant difference between scans (148.0 ± 16.2 min and 143.5 ± 16.6 min, p = 0.52). No patient reported any symptom or medication change between scans.

Mean TBR values are given in Figure 1. The carotid arteries both had significantly higher TBR values than all aortic territories (scan 1 data: carotid vs. ascending aorta $p < 0.05$, vs. arch $p < 0.005$, vs. descending aorta $p < 0.0005$, and vs. abdominal aorta $p < 0.001$). There was no significant difference between the left and right carotid arteries (scan 1: left carotid TBR 1.55 ± 0.28, right carotid TBR 1.64 ± 0.25, p = 0.44). The carotid TBR values are used for sample size calculations in the discussion.

Table 2 Intraclass Correlation Coefficient Values (95% Confidence Intervals in Parentheses)

	Ascending Aorta	Arch of Aorta	Descending Aorta	Abdominal Aorta	Left Carotid	Right Carotid
Interobserver agreement	0.92 (0.70–0.98)	0.71 (0.05–0.92)	0.97 (0.88–1.00)	0.90 (0.10–0.98)	0.97 (0.89–0.99)	0.97 (0.91–0.99)
Intraobserver agreement	0.98 (0.94–1.00)	0.96 (0.85–0.99)	0.93 (0.77–0.98)	0.94 (0.81–0.98)	0.95 (0.83–0.99)	0.98 (0.93–0.99)
Interscan variability	0.92 (0.75–0.98)	0.91 (0.72–0.98)	0.80 (0.43–0.94)	0.79 (0.40–0.93)	0.90 (0.70–0.97)	0.90 (0.68–0.97)

Table 1 shows interscan variability data and gives mean TBR values for all arterial territories for scans 1 and 2, 2 weeks apart, along with the TBR differences and estimated 95% normal ranges for change. It is clear that there is very little spontaneous variation in FDG signal over 2 weeks. This is illustrated in Figure 2, with carotid and aortic images of 2 patients in whom little appreciable change in FDG uptake can be seen between scan 1 and scan 2.

Table 2 shows the ICCs, with 95% confidence intervals for interobserver, intraobserver, and interscan variability. All ICC values except 2 are >0.8 (an accepted marker of excellent agreement [10]), with generally narrow confidence intervals.

Bland-Altman plots demonstrated no evidence of either fixed or proportional bias. Figure 3 shows plots for the right carotid artery. Most of the data points fall within the narrow limits of agreement.

Discussion

We prospectively tested the reproducibility of FDG-PET inflammation imaging in atherosclerosis and showed high reproducibility over 2 weeks in the aorta and carotid arteries. Additionally, inter- and intraobserver agreement measurements are favorable, with the exception of the aortic arch. This territory had only moderate interobserver agreement, likely because of its complex geometry, making accurate placement of similar ROIs difficult for independent readers. We recommend using the carotid artery or ascending aorta in future studies; these areas had the best reproducibility and agreement statistics.

We provided mean and SD values for aorta and carotid TBR values. This information makes it possible to estimate subject numbers for drug trials. An illustration of sample sizes required at different levels of estimated drug effects is provided in Figure 4, based on right carotid TBR data from scan 1. As a result of low standard deviation values, PET imaging requires few subjects to show significant differences between groups. These estimates should be interpreted with caution, however, because they assume a perfect agreement between TBR signal change and underlying change in plaque inflammation, which may not be the case. Nevertheless, they do provide a framework for minimum sample sizes.

We also measured the interscan TBR differences over 2 weeks and established normal ranges of TBR variability for each arterial territory (twice the standard deviation of the difference between scan 1 and scan 2); any change of TBR outside this limit after a therapeutic intervention may be interpreted as a drug effect. For example, in a hypothetical PET/CT trial of a novel anti-inflammatory atherosclerosis therapy, a change in mean TBR in the ascending aorta of 0.14 or more between scan 1 and scan 2 would occur by chance <5% of the time, and we could be 95% confident that it was occurring as a result of a drug effect.

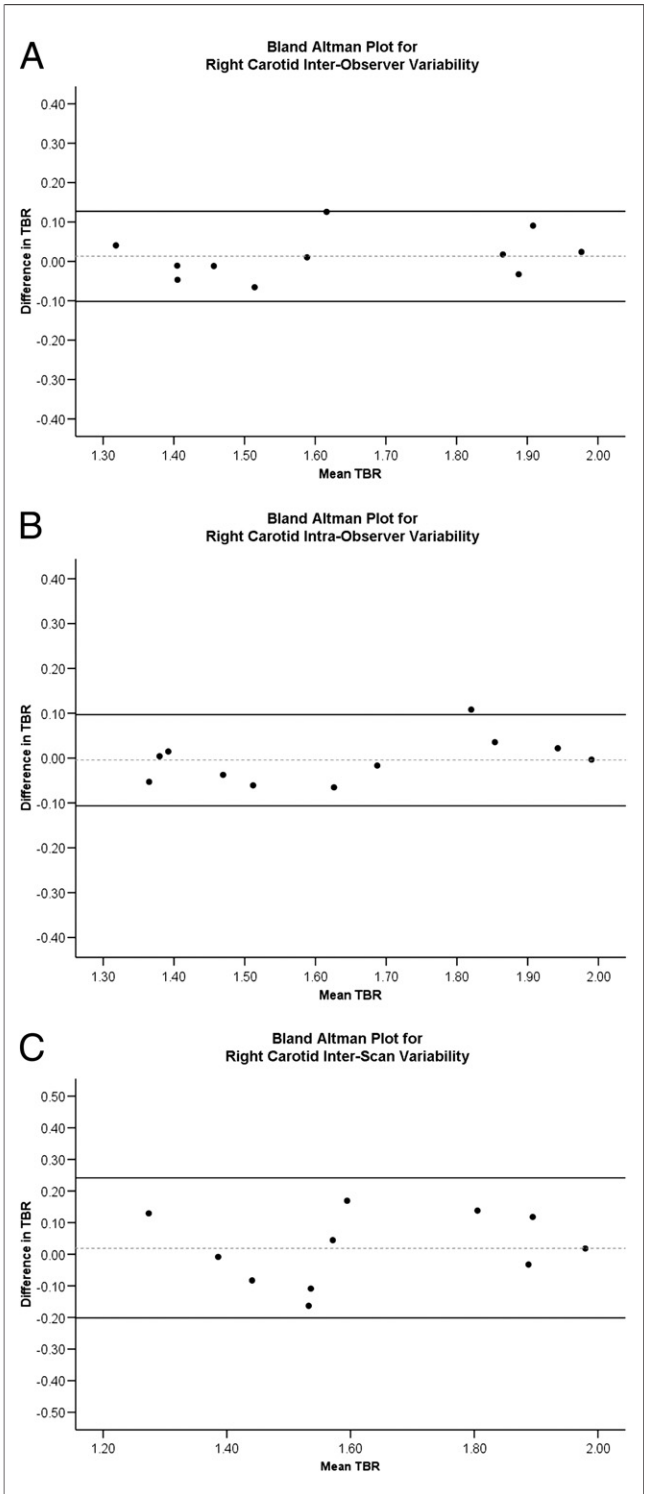

Figure 3 Bland-Altman Plots of Interobserver, Intraobserver, and Interscan Variability

(A) Interobserver, **(B)** intraobserver, and **(C)** interscan variability. The **dashed line** is the mean difference between the 2 scans, while the limits of agreement are drawn as solid lines (mean difference ± 2 SD). TBR = tissue-to-background ratio.

Study limitations Some of the difference in TBR between carotid and aorta could be accounted for by the different scan acquisition times, although this effect is likely to be

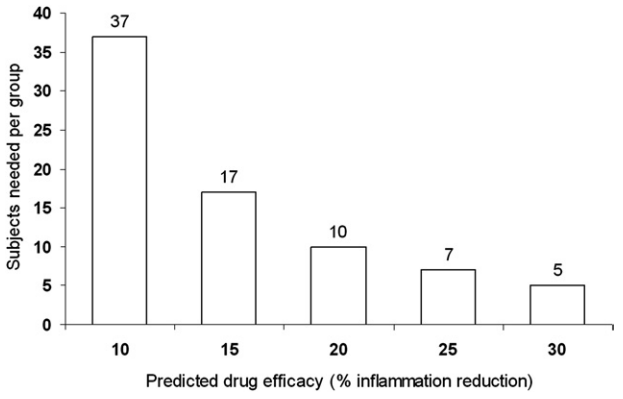

Figure 4 **Sample Size Estimates Based on Drug Efficacy**

The number of subjects needed in trials using ^{18}fluorodeoxyglucose positron emission tomography is shown for several levels of drug effect.

small because of the small absolute values of the TBR measurements.

Comparison with other techniques. These PET reproducibility measurements compare well with other atherosclerosis imaging techniques such as magnetic resonance imaging (MRI) (11), intravascular ultrasound (12), and CT (13). Additionally, the high sensitivity of FDG-PET allows detection of small metabolic changes within plaque that occur before structural alterations can be detected by other modalities. For example, a recent study administered simvastatin 40 mg daily to reduce plaque inflammation and compared it with diet alone in oncology patients. We found that FDG-PET detected the positive effect of therapy within 3 months (8). However, using the same drug in a higher dose, another group imaged plaque area regression with MRI (14). These investigators were not able to see an effect by MRI until 12 months.

Conclusions

We have shown that FDG-PET meets 2 important criteria for serial atherosclerotic plaque imaging. First, spontaneous changes in plaque FDG uptake are low over 2 weeks. Second, interobserver agreement is excellent, meaning that longitudinal multicenter trials of drugs are now feasible.

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